SCIENTIFIC ABSTRACT

Colorectal cancer (CRC) strikes approximately 150,000 patients per year in the United States, with greater than 40% of these patients destined to die of the disease despite current medical management. Death is commonly due to liver metastases with sequelae including progressive liver dysfunction. Hepatocellular cancer (HCC) strikes approximately 8,000 patients per year in the United States and about 1.25 million worldwide. Most patients present with tumors that are unresectable and incurable with existing therapies. The median survival for CRC patients after diagnosis with liver metastases, and for HCC patients, is approximately 6 months or less.

The human p53 gene is a tumor suppressor gene involved in the control of cell proliferation. Loss of wild-type p53 function is associated with the uncontrolled growth of many types of human cancers. The reintroduction and expression of wild-type p53 into p53-altered tumor cells has been shown to suppress tumor growth or induce apoptosis in both *in vitro* and *in vivo* models. It is estimated that greater than 50% of CRC tumors and between 30 to 50% of all HCC tumors have p53 alterations.

This study seeks to evaluate the safety, biological efficacy and the effect of dose of ACN53 treatment. ACN53 is a recombinant adenoviral vector containing the wild-type human p53 gene. ACN53 will be administered by infusion via the hepatic artery, for the regional gene therapy of malignant liver tumors. Study patients will have incurable metastatic (CRC) malignant tumors of the liver or primary (HCC) cancer, with evidence of p53 alteration in their liver tumors. *In vitro* studies have demonstrated p53-specific antiproliferative effects of ACN53 on human HCC and CRC cells and *in vivo* studies have demonstrated p53-specific antiproliferative effects of ACN53 on human CRC cells.

The ACN53 construct is a recombinant, replication-defective, adenovirus derived from adenovirus serotype 5 (Ad5), subgroup C. The adenoviral E1a, E1b and protein IX coding sequences are deleted and replaced with the p53 expression cassette. The deleted E1 region is necessary for viral replication. The virus is additionally deleted for 1.9 kb of DNA sequence in Early Region 3, including that sequence encoding the gp19K protein. The p53 expression cassette contains the human cytomegalovirus immediate early promoter-enhancer, the adenovirus type 2 tripartite leader sequence and a sequence encoding wild-type p53 protein. The human cytomegalovirus immediate early promoter-enhancer directs robust gene expression and the adenovirus type 2 tripartite leader sequence enhances translational efficiency. Polyadenylation is regulated by the E1b and pIX polyadenylation signal. All other regulatory elements and replication origins within ACN53 are endogenous to Ad5. The recombinant adenovirus is similar to other adenoviral vectors reviewed by the RAC and the FDA except that it contains the additional deletion of the pIX coding sequence. The deletion in the pIX coding sequence is expected to reduce the frequency of replication competent adenovirus arising during virus production by reducing the sequence identity with the E1 sequences of the 293 production cell line.

The study design is an open-label, non-randomized, single-dose, dose escalation Phase I clinical trial anticipated to involve a maximum of 27 patients. ACN53 will be administered in escalating doses to successive cohorts of patients until the maximum tolerated dose is determined. Regional ACN53 therapy will be administered as a single bolus infusion via hepatic artery catheter. The route of administration of ACN53 via hepatic artery infusion is designed to maximize gene therapy exposure to the malignant tumors while minimizing exposure to normal tissues outside the liver. The clinical protocol is designed to monitor treatment toxicity. Another objective is to evaluate the biological efficacy, including efficiency and stability of gene transfer by analysis of tumor tissues following therapy. As an important part of this objective the pharmacokinetics of ACN53 will be studied. Clinical evidence of anti-tumor efficacy will also be collected. In addition, the safety and efficacy of different doses levels of ACN53 will be studied.